§ 448.23 Cyclosporine.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cyclosporine is a cyclic polypeptide consisting of 11 amino acids. It is a white or essentially white finely crystalline powder. It is so purified and dried that:

(i) Its cyclosporine content is not less than 975 micrograms per milligram and not more than 1,020 micrograms per milligram on the anhydrous basis.

(ii) Its loss on drying is not more than 3.0 percent.

(iii) Its heavy metals content is not more than 20 parts per million.

(iv) It passes the identity test.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for cyclosporine content, loss on drying, heavy metals, and identity.

(ii) Samples, if required by the Director, Center for Drug Evaluation and Research: 10 packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Cyclosporine content.* Proceed as directed in § 436.346 of this chapter, except prepare the working standard and sample solutions and calculate the cyclosporine content as described in paragraphs (b)(1) (i) and (ii) of this section. A typically suitable column for cyclosporine is a 250-millimeter column having an inside diameter of 4 millimeters packed with octyl silane chemically bonded to totally porous microsilica particles, 5 to 7 microns in diameter.

(i) *Preparation of working standard and sample solutions.*

NOTE: Dissolve working standards and samples immediately before analysis.

(*a*) *Preparation of working standard solution.* Dissolve an accurately weighed portion of the working standard in ethanol by shaking for at least 15 minutes. If necessary, ultrasonicate until the solution becomes completely clear. Dilute with ethanol to obtain a solution containing 1,000 micrograms of cyclosporine activity per milliliter.

(*b*) *Preparation of sample solutions.* Prepare all sample solutions as directed for preparation of working standard solutions, except dilute with ethanol to obtain a solution containing 1,000 micrograms of cyclosporine per milliliter (estimated).

(ii) *Calculations.* Calculate the micrograms of cyclosporine per milligram of sample as follows:

$$\text{Micrograms of cyclosporine per milligram} = \frac{A_u \times P_s \times 100}{A_s \times C_u \times (100 - m)}$$

where:

A_u=Area of the cyclosporine peak in the chromatogram of the sample (at a retention time equal to that observed for the standard);

A_s=Area of the cyclosporine peak in the chromatogram of the cyclosporine working standard;

P_s=Cyclosporine activity in the cyclosporine working standard solution in micrograms per milliliter;

C_u=Milligrams of cyclosporine per milliliter of sample solution; and

m=Percent loss on drying of the sample.

(2) *Loss on drying.* Proceed as directed in § 436.200(a) of this chapter.

(3) *Heavy metals.* Proceed as directed in § 436.208 of this chapter.

(4) *Identity.* The high-pressure liquid chromatogram of the sample determined as directed in paragraphs (b)(1) of this section compares qualitatively to that of the cyclosporine working standard.

[49 FR 22632, May 31, 1984, as amended at 55 FR 11584, Mar. 29, 1990]

§ 448.25 Gramicidin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Gramicidin is the white, or nearly white, odorless, crystalline compound of a kind of gramicidin or a mixture of two or more such compounds. It is so purified and dried that:

(i) It has a potency of not less than 900 micrograms of gramicidin per milligram.

(ii) [Reserved]

(iii) Its loss on drying is not more than 3 percent.

(iv) Its residue on ignition is not more than 1.0 percent.